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# A pyrrolidine-based specific inhibitor of cytosolic phospholipase A<sub>2</sub>α blocks arachidonic acid release in a variety of mammalian cells

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John Turk <sup>c</sup>, Christina C. Leslie <sup>b</sup>, Michael H. Gelb <sup>a,\*</sup>

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## Abstract

We analyzed a recently reported (K. Seno, T. Okuno, K. Nishi, Y. Murakami, F. Watanabe, T. Matsuura, M. Wada, Y. Fujii, M. Yamada, T. Ogawa, T. Okada, H. Hashizume, M. Kii, S.-H. Hara, S. Hagishita, S. Nakamoto, *J. Med. Chem.* 43 (2000)) pyrrolidine-based inhibitor, pyrrolidine-1, against the human group IV cytosolic phospholipase A<sub>2</sub> α-isoform (cPLA<sub>2</sub>α). Pyrrolidine-1 inhibits cPLA<sub>2</sub>α by 50% when present at approx. 0.002 mole fraction in the interface in a number of in vitro assays. It is much less potent on the cPLA<sub>2</sub>γ isoform, calcium-independent group VI PLA<sub>2</sub> and groups IIA, X, and V secreted PLA<sub>2</sub>s. Pyrrolidine-1 blocked all of the arachidonic acid released in Ca<sup>2+</sup> ionophore-stimulated CHO cells stably transfected with cPLA<sub>2</sub>α, in zymosan- and okadaic acid-stimulated mouse peritoneal macrophages, and in ATP- and Ca<sup>2+</sup> ionophore-stimulated MDCK cells. © 2001 Elsevier Science B.V. All rights reserved.

**Keywords:** Cystolic phospholipase; Arachidonic acid; Pyrrolidine-based inhibitor

## 1. Introduction

Phospholipase A<sub>2</sub> (PLA<sub>2</sub>) catalyzes the hydrolysis of the *sn*-2 ester of glycerophospholipids to release a

fatty acid and a lysophospholipid. Mammalian PLA<sub>2</sub>s comprise a superfamily of enzymes. Nine different secreted PLA<sub>2</sub>s have been identified in humans [1–4]. These small (approx. 16 kDa), disulfide-rich enzymes require millimolar calcium for catalytic activity, and are secreted by the classical secretory pathway in response to proinflammatory cytokines or by degranulation [5]. Functions of secreted PLA<sub>2</sub>s include arachidonate release for eicosanoid production [6] and bactericidal activity [7].

An 87 kDa group IV cytosolic phospholipase A<sub>2</sub> α-isoform (cPLA<sub>2</sub>α) uses its C2 domain to translocate to the perinuclear region of cells in response to a rise in intracellular calcium [8,9]. This enzyme mediates agonist-induced arachidonic acid release in a

Abbreviations: BEL, (*E*)-6-(bromomethylene)tetrahydro-3-(1-naphthalenyl)-2*H*-pyran-2-one; BSA, bovine serum albumin; cPLA<sub>2</sub>α and cPLA<sub>2</sub>γ, human cytosolic phospholipase A<sub>2</sub> α- and γ-isoforms; DOPM, 1,2-dioleoylphosphatidylmethanol; GLU, ester formed between γ-linolenic acid and umbelliferone; iPLA<sub>2</sub>β and iPLA<sub>2</sub>γ, calcium-independent phospholipases A<sub>2</sub> β- and γ-isoforms; PLA<sub>2</sub>, phospholipase A<sub>2</sub>

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variety of mammalian cells [10]. The  $\beta$ - and  $\gamma$ -isoforms of cPLA<sub>2</sub> have been recently identified [11,12], but their physiological functions are unknown.

A second cytosolic PLA<sub>2</sub> has been cloned [13–15] that does not require Ca<sup>2+</sup> for catalysis. It is classified as a group VIA PLA<sub>2</sub> and is designated iPLA<sub>2</sub> $\beta$  [16,17] to distinguish it from a membrane-associated, Ca<sup>2+</sup>-independent PLA<sub>2</sub> that contains a peroxisomal targeting sequence and is designated iPLA<sub>2</sub> $\gamma$ . These enzymes contain a GXSXG lipase consensus motif and are inhibited by the mechanism-based inactivator (*E*)-6-(bromomethylene)tetrahydro-3-(1-naphthalenyl)-2*H*-pyran-2-one (BEL) [18,19].

PLA<sub>2</sub> inhibitors that are specific for each of the superfamily members and are cell permeable are useful tools for understanding the role of these enzymes in cellular processes. Key issues for the proper analysis of PLA<sub>2</sub> inhibitors have been reviewed elsewhere [20,21], and a recent review of active site-directed PLA<sub>2</sub> inhibitors has appeared [22]. Cell permeable and potent inhibitors of cPLA<sub>2</sub> $\alpha$  include the analogues of arachidonic acid with the COOH group replaced with COCF<sub>3</sub> (AACOCF<sub>3</sub> [23]) or PO(O-Me)F (MAFP [24]). These first-generation cPLA<sub>2</sub> $\alpha$  inhibitors have been extremely useful in probing the role of this enzyme in cellular processes including arachidonic acid release in thrombin-stimulated platelets [24–26]. However, these arachidonic acid analogues block enzymes in the eicosanoid pathway that oxygenate arachidonate [26], and they also inhibit iPLA<sub>2</sub> $\beta$  [27].

Recently a class of pyrrolidine-containing compounds have been reported to act as potent inhibitors of cPLA<sub>2</sub> $\alpha$  in vitro and to block arachidonate release in calcium ionophore-stimulated THP-1 cells

[28]. One of the most potent compounds in the series, designated pyrrolidine-1 in the present study, is shown in Fig. 1. Here, we report that low- and sub-micromolar concentrations of pyrrolidine-1 block the action of cPLA<sub>2</sub> $\alpha$  in a number of different in vitro assays and are much less potent on other PLA<sub>2</sub> family members (cPLA<sub>2</sub> $\gamma$ , iPLA<sub>2</sub> $\beta$  and human groups IIA, V, and X secreted PLA<sub>2</sub>s). We also report that low- and sub-micromolar concentrations of pyrrolidine-1 block arachidonate acid liberation from membrane phospholipids in a number of different mammalian cells stimulated with a diverse array of agonists.

## 2. Materials and methods

### 2.1. Materials

Pyrrolidine-1 was synthesized as described previously [29]. Its structure was confirmed by <sup>1</sup>H-NMR and electrospray mass spectrometry. The compound was judged to be pure by thin layer chromatography on a silica plate and by HPLC on a reverse-phase (C18) column. Other materials were obtained as follows: GLU [30], BEL (Cayman Chemicals), phospholipids (Avanti), radiolabeled phospholipids (NEN), 6His-tagged cPLA<sub>2</sub> $\alpha$  [31], 6His-tagged cPLA<sub>2</sub> $\gamma$  (submitted for publication), iPLA<sub>2</sub> $\beta$  [32], human groups IIA, X and V secreted PLA<sub>2</sub>s [33,34] (to be published).

### 2.2. In vitro inhibition studies

For all assays, pyrrolidine-1 was added from a stock solution in DMSO such that the final concentration of DMSO in the assay did not exceed 1%. The effect of the pyrrolidine-1 on PLA<sub>2</sub> activity of purified cPLA<sub>2</sub> $\alpha$  (40 ng) and cPLA<sub>2</sub> $\gamma$  (4 ng) was tested using 30  $\mu$ M 1-palmitoyl-2-[<sup>14</sup>C]arachidonylphosphatidylcholine co-sonicated with 9  $\mu$ M dioleoylglycerol. Assays (50  $\mu$ l) were carried out in 50 mM HEPES, pH 7.4, 1 mM EGTA, 150 mM NaCl and either 2  $\mu$ M cPLA<sub>2</sub> $\gamma$  or 1  $\mu$ M cPLA<sub>2</sub> $\alpha$ . Inhibitor was added and reactions started by the addition of enzyme. After incubation at 37°C for 2 min, reactions were stopped by the addition of Dole's reagent, and fatty acids extracted as described previously [35].

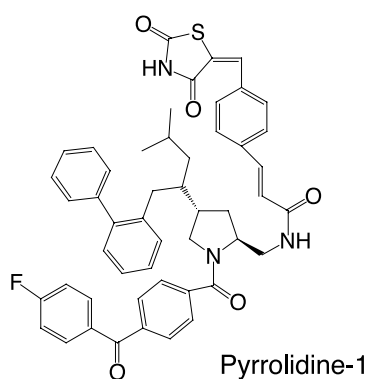


Fig. 1. Structure of pyrrolidine-1.

Pyrrolidine-1 was also tested as a cPLA<sub>2</sub>α inhibitor using a 1 ml mixed-micelle assay (50 mM Tris, pH 8.0, 150 mM NaCl, 0.3 mM CaCl<sub>2</sub>, 0.2 mM EGTA, 30% glycerol (v/v), 0.024% Triton X-100 (w/v), 100 μM 1,2-dioleoylphosphatidylmethanol (DOPM), 2.8 μM GLU)) and a 1 ml vesicle assay (50 mM Tris, pH 8.0, 0.3 mM CaCl<sub>2</sub>, 0.2 mM EGTA, 30% glycerol (v/v), 100 μM DOPM (0.1 μm extruded vesicles)) as described [30].

cPLA<sub>2</sub>γ was also assayed with [<sup>3</sup>H]arachidonic acid-labeled Sf9 cells expressing cPLA<sub>2</sub>γ in which endogenous membrane phospholipids are used as substrate. Sf9 cells (6 × 10<sup>6</sup> cells per 100 mm dish) were infected with control or recombinant viruses. After 30 h, cells were labeled with 0.5 μCi/ml [<sup>3</sup>H]arachidonic acid for 18 h in medium containing 10% FBS. Cells were washed twice with PBS containing 0.01% fatty acid-free bovine serum albumin (BSA) and then once in ice-cold homogenization buffer (50 mM HEPES, pH 7.4, 100 mM KCl, 5 mM NaCl, 1 mM EGTA, 10 μg/ml pepstatin A, 16 μg/ml benzamidine HCl and 10 μg/ml phenanthroline). Cells were scraped in homogenization buffer and sonicated three times (15 s each) on ice. Lysates were centrifuged at 100 000 × g at 4°C for 1 h. Membranes were suspended in homogenization buffer containing 5 mM MgCl<sub>2</sub>, 1 mg/ml fatty acid-free BSA and 2 μM CaCl<sub>2</sub> and pyrrolidine-1. Samples were incubated at 37°C for 10 min. Lipids were extracted by the Bligh and Dyer method and free fatty acids separated by on a silica plate TLC (oleic acid standard) with hexane:ether:acetic acid (80:20:2). The fatty acid region was scraped and submitted to scintillation counting.

iPLA<sub>2</sub>β was incubated for 3 min at room temperature with BEL (added from an ethanol stock solution) and at 37°C for 2 min with pyrrolidine-1 in 40 mM Tris, pH 7.3, 5 mM EGTA. Five microliters of 1-palmitoyl-2-(1-[<sup>14</sup>C]linoleoyl)phosphatidylcholine in ethanol were added (2.5 μM, 55 Ci/mol). After a 5 min incubation at 37°C, 100 μl of butanol was added. After centrifugation, 25 μl of the upper layer was co-spotted with oleic acid on a silica gel G TLC plate which was developed with petroleum ether:ether:acetic acid (80:20:1). The fatty acid region (I<sub>2</sub> vapor) was scraped and submitted to scintillation counting.

Human groups IIA, V, and X were assayed with 30 μM 1-palmitoyl-2-oleoylphosphatidylglycerol vesicles (0.1 micron [30]) in Hanks' balanced salt so-

lution with 1 mM CaCl<sub>2</sub> and MgCl<sub>2</sub> at 37°C using the fatty acid binding protein assay [34].

### 2.3. Studies with cultured mammalian cells

CHO cells stably transfected with wild-type cPLA<sub>2</sub>α, radiolabeling with [<sup>3</sup>H]arachidonic acid, and A23187-dependent arachidonate release have been described previously [36]. Cells were preincubated in complete culture medium containing various concentrations of pyrrolidine-1 for 15 min and with 1 μM PMA for 10 min prior to stimulation with 1 or 10 μM A23187 for 20 min (37°C).

MDCK cells were plated at 1.2 × 10<sup>6</sup> cells per well in 12-well tissue culture plates in DMEM containing 10% FBS and incubated overnight. Cells were washed twice with Hanks' buffered saline solution and then incubated overnight with 0.25 μCi/well [<sup>3</sup>H]arachidonic acid (98 Ci/mmol) in DMEM containing 0.2% BSA.

Mouse peritoneal macrophages were harvested and plated at 1 × 10<sup>6</sup> cells per well in 24-well tissue culture plates in DMEM containing 10% FBS as previously described [35,37,38]. Cells were allowed to attach for 2 h at 37°C and then washed twice with Hanks' balanced salt solution, before being labeled overnight with 0.2 μCi/well [<sup>3</sup>H]arachidonic acid in DMEM containing 10% FBS.

After labeling, MDCK and macrophages were washed three times with DMEM containing 0.05% BSA and then preincubated with the indicated inhibitor concentrations for 30 min at 37°C. MDCK cells were stimulated for 3 min with either 100 nM ATP or 1 μM ionomycin. Mouse peritoneal macrophages were stimulated either with zymosan (10 particles per cell) for 60 min or 1 μM okadaic acid for 90 min. Radioactivity released into the supernatant was counted and calculated as the percentage of total radioactivity (cellular and released). Results are represented as a percentage of maximum response and are representative of three individual experiments.

## 3. Results and discussion

### 3.1. *In vitro* PLA<sub>2</sub> inhibition studies

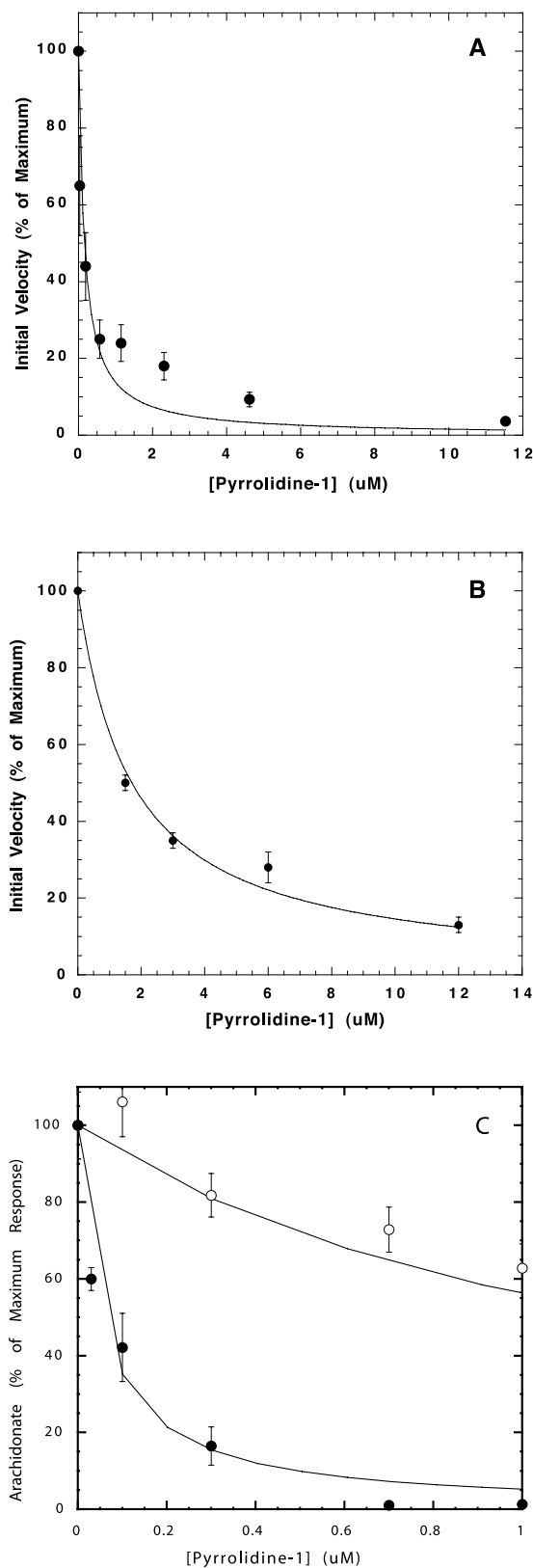
Pyrrolidine-1 was tested as an inhibitor of cPLA<sub>2</sub>α using a number of different *in vitro* assays. Previous

Fig. 2. Inhibition of cPLA<sub>2</sub>α by pyrrolidine-1 measured with the DOPM/GLU vesicle assay (A) and the Triton X-100/DOPM/GLU mixed-micelle assay (B). C shows the inhibition of cPLA<sub>2</sub>α (●) and cPLA<sub>2</sub>γ (○) measured with the 1-palmitoyl-2-arachidonylphosphatidylcholine/1,2-dioleoylglycerol vesicle assay. Error bars are the standard deviations determined from 2–3 independent assays. Other conditions are given in Section 2. Solid lines in A and B are the theoretical inhibition curves for competitive inhibition. Average values and standard errors for 2–3 independent experiments are shown.

studies have shown that cPLA<sub>2</sub>α binds tightly to anionic DOPM vesicles [30,31] and to zwitterionic phosphatidylcholine vesicles [31,39,40] and displays similar specific activity on both [31]. The fatty acid ester GLU is hydrolyzed by cPLA<sub>2</sub>α to release umbelliferone, which is detected in a real-time fluorimetric assay [30]. Fig. 2A shows that pyrrolidine-1 is a potent inhibitor of cPLA<sub>2</sub>α-catalyzed hydrolysis of GLU present in DOPM vesicles, displaying an IC<sub>50</sub> in this assay of 0.18 μM (0.0018 mole fraction in the interface).

As shown in Fig. 2B, the same compound also is a potent cPLA<sub>2</sub>α inhibitor, IC<sub>50</sub> = 1.8 μM (approx. 0.002 mole fraction in the interface), when analyzed with a mixed-micelle assay composed of GLU in Triton X-100 micelles containing DOPM to facilitate interfacial binding of enzyme [30]. As shown in Fig. 2C, pyrrolidine-1 also displays potent inhibition of cPLA<sub>2</sub>α acting on 1-palmitoyl-2-arachidonylphosphatidylcholine vesicles containing 23 mole% 1,2-dioleoylglycerol (IC<sub>50</sub> = 0.07 μM, 0.0017 mole fraction in the interface). Diglyceride leads to an increase in cPLA<sub>2</sub>α activity on phosphatidylcholine vesicles [41,42].

It is difficult to compare the relative IC<sub>50</sub> values for pyrrolidine-1 obtained with the three different *in vitro* cPLA<sub>2</sub>α assays. This is because with the different interface concentrations, the surface concentration of inhibitor, which determines the degree of inhibition, is different in the different assays. Also the surface concentration of substrates and the interfacial *K<sub>m</sub>* values are different in the different assays. Finally, each of the diluent amphiphiles, DOPM, Triton X-100, 1,2-dioleoylglycerol, competes to different extents with pyrrolidine-1 for the binding to cPLA<sub>2</sub>α. However, it is clear that pyrrolidine-1 is a potent cPLA<sub>2</sub>α inhibitor since it inhibits when



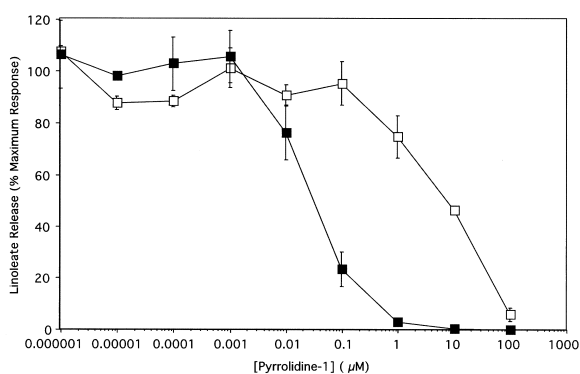


Fig. 3. Inhibition of  $iPLA_2\beta$  by BEL (■) and pyrrolidine-1 (□) measured with the 1-palmitoyl-2-linoleoylphosphatidylcholine assay. Other conditions are given in Section 2. Average values and standard errors for 2–3 independent experiments are shown.

present in the substrate-containing interface at a mole fraction of approx. 0.002, i.e. one inhibitor per 500 interface amphiphiles. Thus, it is clear that this compound inhibits  $cPLA_2\alpha$  by a specific mechanism involving protein–inhibitor interaction [20,21].

In contrast, under similar assay conditions pyrrolidine-1 is 17-fold less potent on purified  $cPLA_2\gamma$  ( $IC_{50} = 1.2 \mu M$ ) than on  $cPLA_2\alpha$  (Fig. 2C). Unlike  $cPLA_2\alpha$ ,  $cPLA_2\gamma$  is constitutively associated with membranes in cells. Using isolated membranes from Sf9 cells expressing  $cPLA_2\gamma$ , in which endogenous membrane phospholipid is used as substrate by membrane-associated  $cPLA_2\gamma$ , pyrrolidine-1 ( $\leq 10 \mu M$ ) did not inhibit the activity of  $cPLA_2\gamma$  (data not shown). This suggests that under more physiological conditions, pyrrolidine-1 does not inhibit  $cPLA_2\gamma$ .

As shown in Fig. 3, pyrrolidine-1 inhibits calcium-independent cytosolic phospholipase  $A_2$  ( $iPLA_2\beta$ ) with an  $IC_{50}$  of  $8 \mu M$  in an assay with  $2.5 \mu M$  phosphatidylcholine substrate. This is almost certainly due to a non-specific physical effect of pyrrolidine-1 on the structure of the substrate vesicles since the mole fraction of inhibitor in the interface is approx. 0.8. Inhibition of an interfacial enzyme under conditions of  $[I] > [S]$  cannot be interpreted as specific inhibition [20,21]. In the same assay, the well-established  $iPLA_2\beta$  inhibitor BEL [19] displays an  $IC_{50}$  of  $30 nM$  (mole fraction in the interface of 0.012).

No detectable inhibition of the three secreted phospholipase  $A_2$  human groups IIA, V, and X were observed when up to  $10 \mu M$  pyrrolidine-1 was added to a fluorimetric assay with  $30 \mu M$  1-palmitoyl-2-oleoylphosphatidylglycerol vesicles (not shown).

### 3.2. Mammalian cell studies with pyrrolidine-1

As a first step in evaluating pyrrolidine-1 as an inhibitor of  $cPLA_2\alpha$  action in mammalian cells, we carried out studies with CHO cells stably overexpressing  $cPLA_2\alpha$  [36]. Arachidonate release in  $cPLA_2\alpha$ -transfected cells minus that released from non-transfected cells is taken as release that is  $cPLA_2\alpha$ -dependent. Thus, the transfected CHO cell system provides an ideal setting for testing pyrrolidine-1 as an inhibitor of  $cPLA_2\alpha$  in vivo. As shown in Fig. 4, pyrrolidine-1 is a potent inhibitor of CHO cell arachidonate release induced by  $1 \mu M$  and  $10 \mu M$  A23187 in the presence of  $1 \mu M$  PMA, displaying an  $IC_{50}$  of  $0.2$ – $0.5 \mu M$  in both cases. Inhibition approaches 100% with  $4$ – $10 \mu M$  pyrrolidine-1.

Studies with peritoneal macrophages from mouse containing a targeted disruption of the  $cPLA_2\alpha$  gene have shown that virtually all of the arachidonate release in response to the agonists zymosan and okadaic acid is dependent on  $cPLA_2\alpha$  [38]. Zymosan (yeast cell walls) induces a transient calcium increase and mitogen-activated protein kinase activation, which together fully activate  $cPLA_2\alpha$ . In contrast, okadaic acid induces  $cPLA_2\alpha$ -mediated arachidonic acid release by unknown mechanisms since it occurs

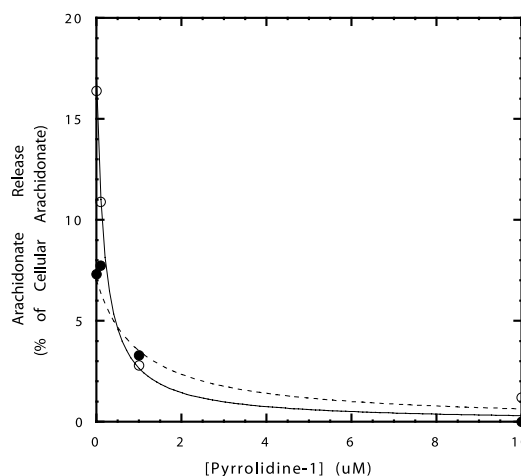


Fig. 4. Inhibition of arachidonate release in CHO cells that are stably transfected with  $cPLA_2\alpha$ . Release of arachidonate was triggered by  $1 \mu M$  (●) or  $10 \mu M$  (○) concentrations of the  $Ca^{2+}$  ionophore A23187 in the presence of  $1 \mu M$  PMA. Plotted is the percentage of total cell-incorporated radiolabeled arachidonate released in  $cPLA_2\alpha$ -transfected cells minus that which was released from non-transferred parental CHO cells. Other conditions are given in Section 2. Average values and standard errors for 2–3 independent experiments are shown.

without an increase in intracellular calcium but is dependent on activation of mitogen-activated protein kinase. As shown in Fig. 5, pyrrolidine-1 blocks virtually all of the arachidonate released in response to zymosan and okadaic acid, with  $IC_{50}$  values of 0.1 and 0.2  $\mu$ M, respectively.

We also studied the effect of pyrrolidine-1 on arachidonate release from MDCK cells. In MDCK cells, cPLA<sub>2</sub> $\alpha$ -mediated arachidonic acid release occurs through P2Y<sub>2</sub> receptor activation by ATP that requires an IP<sub>3</sub>-mediated increase in intracellular calcium [43]. The MDCK cell model has recently been used to study the role of calcium and the C2 domain in regulating the translocation of cPLA<sub>2</sub> $\alpha$  to specific membranes in response to ATP and ionomycin (submitted for publication). As shown in Fig. 6, pyrrolidine-1 inhibited virtually all of the arachidonate released by these two agonists with an  $IC_{50}$  of 0.8  $\mu$ M in both cases.

### 3.3. Concluding remarks

Pyrrolidine-1 displays potent inhibition of cPLA<sub>2</sub> $\alpha$  in the sub-micromolar range when analyzed with a number of in vitro assays. It inhibits cPLA<sub>2</sub> $\gamma$  and iPLA<sub>2</sub> $\beta$  only at very high concentrations and probably by a non-specific mechanism, and it does not inhibit secreted PLA<sub>2</sub>s. Given that this compound is

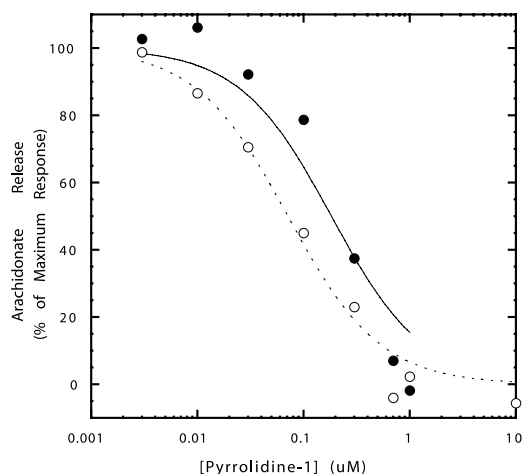


Fig. 5. Inhibition of arachidonate release in mouse peritoneal macrophages induced by zymosan (○) or okadaic acid (●). Plotted is the percentage of maximal arachidonate released in the absence of pyrrolidine-1. Other conditions are given in Section 2. Average values and standard errors for 2–3 independent experiments are shown.

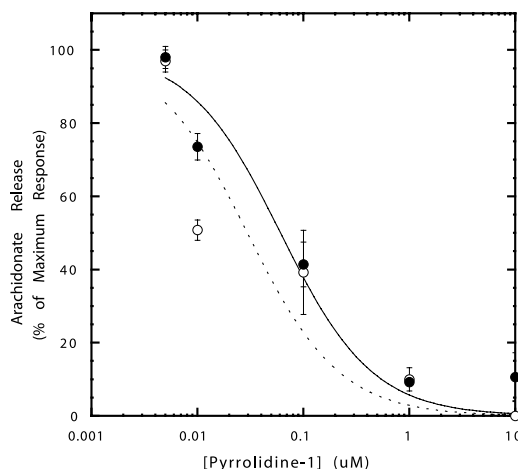


Fig. 6. Inhibition of arachidonate release in MDCK cells induced by ATP (○) or the Ca<sup>2+</sup> ionophore ionomycin (●). Plotted is the percentage of maximal arachidonate released in the absence of pyrrolidine-1. Other conditions are given in Section 2. Average values and standard errors for 2–3 independent experiments are shown.

charge neutral and contains a considerable amount of hydrophobic substituents, it is not surprising that it penetrates a number of mammalian cells, as judged by its ability to block cPLA<sub>2</sub> $\alpha$ -dependent arachidonate release in the sub- to low-micromolar range. A large number of studies have been reported which suggest a role for cPLA<sub>2</sub> $\alpha$  in arachidonate acid release for eicosanoid biosynthesis and other cell processes, for example ion channel modulation [44] and augmentation of capacitative calcium influx [45]. Pyrrolidine-1 should be an extremely useful tool to study cPLA<sub>2</sub> $\alpha$  functions, especially when studies with cPLA<sub>2</sub> $\alpha$ -deficient mice are not practical.

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### References

- [1] E. Valentin, G. Lambeau, *Biochim. Biophys. Acta* 1488 (2000) 59–70.

- [2] M.H. Gelb, E. Valentin, F. Ghomashchi, M. Lazdunski, G. Lambeau, *J. Biol. Chem.* 275 (2000) 39823–39826.
- [3] E. Valentin, A.G. Singer, F. Ghomashchi, M. Lazdunski, M.H. Gelb, G. Lambeau, *Biochem. Biophys. Res. Commun.* 279 (2000) 223–228.
- [4] N. Suzuki, J. Ishizaki, Y. Yokota, K. Higashino, T. Ono, M. Ikeda, N. Fujii, K. Kawamoto, K. Hanasaki, *J. Biol. Chem.* 275 (2000) 5785–5793.
- [5] I. Kudo, M. Murakami, S. Hara, I.K., *Biochim. Biophys. Acta* 117 (1993) 217–231.
- [6] M. Murakami, Y. Nakatani, G. Atsumi, K. Inoue, I. Kudo, *Crit. Rev. Immunol.* 17 (1997) 225–283.
- [7] X.D. Qu, R.I. Lehrer, *Infect. Immun.* 66 (1998) 2791–2797.
- [8] S. Glover, M. de Carvalho, T. Bauburt, M. Jonas, E. Chi, C.C. Leslie, M.H. Gelb, *J. Biol. Chem.* 270 (1995) 15359–15367.
- [9] E.A. Nalefski, J.J. Falke, *Protein Sci.* 5 (1996) 2375–2390.
- [10] C.C. Leslie, *J. Biol. Chem.* 272 (1997) 16709–16712.
- [11] R.T. Pickard, B.A. Striffler, R.M. Kramer, J.D. Sharp, *J. Biol. Chem.* 274 (1999) 8823–8831.
- [12] K.W. Underwood, C. Song, R.W. Kriz, X.J. Chang, J.L. Knopf, *J. Biol. Chem.* 273 (1998) 21926–21932.
- [13] J. Tang, R.W. Kriz, N. Wolfman, M. Shaffer, S. Seehra, S. Jones, *J. Biol. Chem.* 272 (1997) 8567–8575.
- [14] M.A. Balboa, J. Balsinde, S. Jones, E.A. Dennis, *J. Biol. Chem.* 272 (1997) 8576–8590.
- [15] Z. Ma, S. Ramanadham, K. Kempe, X.S. Chi, J.L. Ladenson, *J. Turk. J. Biol. Chem.* 272 (1997) 11118–11127.
- [16] E.A. Dennis, *Trends Biochem. Sci.* 22 (1997) 1–2.
- [17] J. Balsinde, E.A. Dennis, *J. Biol. Chem.* 272 (1997) 16069–16072.
- [18] S.L. Hazen, L.A. Zupan, R.H. Weiss, D.P. Getman, R.W. Gross, *J. Biol. Chem.* 266 (1991) 7227–7232.
- [19] L.A. Zupan, R.H. Weiss, S.L. Hazen, B.L. Parnas, K.W. Aston, P.J. Lennon, D.P. Getman, R.W. Gross, *J. Med. Chem.* 36 (1993) 95–100.
- [20] M.H. Gelb, M.K. Jain, O.G. Berg, *FASEB J.* 8 (1994) 916–924.
- [21] M.K. Jain, M.H. Gelb, J. Rogers, O.G. Berg, *Methods Enzymol.* 249 (1995) 567–614.
- [22] M.H. Gelb, I. Kudo, *Tanpakushitsu Kakusan Koso* 45 (2000) 1065–1071.
- [23] I.P. Street, H.-K. Lin, F. Laliberte, F. Ghomashchi, Z. Wang, H. Perrier, N.M. Tremblay, Z. Huang, P.K. Weech, M.H. Gelb, *Biochemistry* 32 (1993) 5935.
- [24] Z. Huang, S. Liu, I. Street, F. Laliberte, K. Abdullah, S. Desmarais, Z. Wang, B. Kennedy, P. Payette, D. Riendeau, P. Weech, M. Gresser, *Mediators Inflamm.* 3 (1994) 307–308.
- [25] F. Bartoli, H.-K. Lin, F. Ghomashchi, M.H. Gelb, M.K. Jain, R. Apitz-Castro, *J. Biol. Chem.* 269 (1994) 15625–15630.
- [26] D. Riendeau, J. Guay, P.K. Weech, F. Laliberte, J. Yergey, C. Li, S. Desmarais, H. Perrier, S. Liu, D. Nicoll-Griffith, I.P. Street, *J. Biol. Chem.* 269 (1994) 15619–15624.
- [27] F. Ghomashchi, R. Loo, J. Balsinde, F. Bartoli, R. Apitz-Castro, J.D. Clark, E.A. Dennis, M.H. Gelb, *Biochim. Biophys. Acta* 1420 (1999) 45–56.
- [28] K. Seno, T. Okuno, K. Nishi, Y. Murakami, F. Watanabe, T. Matsuura, M. Wada, Y. Fujii, M. Yamada, T. Ogawa, T. Okada, H. Hashizume, M. Kii, S.-H. Hara, S. Hagishita, S. Nakamoto, *J. Med. Chem.* 43 (2000) 1041–1044.
- [29] K. Seno, M. Ohtani, F. Watanabe, H. Tamauchi, *Patent WO98/33797*, 1998.
- [30] T. Bayburt, B.Z. Yu, I. Street, F. Ghomashchi, F. Laliberte, H. Perrier, Z. Wang, R. Homan, M.K. Jain, M.H. Gelb, *Anal. Biochem.* 232 (1995) 7–23.
- [31] M.S. Hixon, A. Ball, M.H. Gelb, *Biochemistry* 37 (1998) 8516.
- [32] M.H. Wolf, R.W. Gross, *J. Biol. Chem.* 271 (1996) 30879–30885.
- [33] Y. Snitko, R.S. Koduri, S.K. Han, R. Othman, S.F. Baker, B. Molini, D.C. Wilton, M.H. Gelb, W. Cho, *Biochemistry* 36 (1997) 14325–14333.
- [34] S. Bezzine, R.S. Koduri, E. Valentin, M. Murakami, I. Kudo, F. Ghomashchi, M. Sadilek, G. Lambeau, M.H. Gelb, *J. Biol. Chem.* 275 (2000) 3179–3191.
- [35] M.G. de Carvalho, J. Garritano, C.C. Leslie, *J. Biol. Chem.* 270 (1995) 20439–20446.
- [36] Y. Hefner, A.G. Börsch-Haubold, J.I. Wilde, S. Pasquels, D. Schieltz, F. Ghomashchi, J.R.I. Yates, C. Armstrong, A. Paterson, P. Cohen, T. Hunter, I. Kudo, S. Watson, M.H. Gelb, *J. Biol. Chem.* 75 (2000) 37542–37551.
- [37] Z.-H. Qiu, C.C. Leslie, *J. Biol. Chem.* 269 (1994) 19480–19487.
- [38] M.A. Gijon, D.M. Spencer, A.R. Siddiqi, J.V. Bonventre, C.C. Leslie, *J. Biol. Chem.* 275 (2000) 46–46.
- [39] M. Mosior, D.A. Six, E.A. Dennis, *J. Biol. Chem.* 273 (1998) 2184–2191.
- [40] E.A. Nalefski, T. McDonagh, W. Somers, J. Seehra, J.J. Falke, J.D. Clark, *J. Biol. Chem.* 273 (1998) 1365–1372.
- [41] R.M. Kramer, G.C. Checani, D. Deykin, *Biochem. J.* 248 (1987) 779–783.
- [42] C.C. Leslie, J.Y. Channon, *Biochim. Biophys. Acta* 1045 (1990) 261–270.
- [43] M. Xing, B. Firestein, G. Shen, P. Insel, *J. Clin. Invest.* 99 (1997) 805–814.
- [44] R. Levy, A. Lowenthal, R. Dana, *Adv. Exp. Med. Biol.* 479 (2000) 125–135.
- [45] J.T. Weber, B.A. Rzigalinski, E.F. Ellis, *J. Biol. Chem.* 276 (2001) 1800–1807.